EasySep™ Human EpCAM Positive Selection Kit II

Positive Selection

Catalog #17846

For processing 1 x 10^9 cells

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Document #DX22520 | Version 1_1_0

Description

Isolate highly purified EpCAM+ cells from fresh or previously frozen cultured human mammary epithelial cells or other dissociated tissue samples by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 99% purity
- No columns required

This kit targets EpCAM+ cells for positive selection with an antibody recognizing the EpCAM surface marker. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human EpCAM Positive Selection Cocktail II	17846C	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS. Includes an Fc receptor blocking antibody.
EasySep™ Dextran RapidSpheres™ 50100	50100	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in water.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

Cells must be in a single-cell suspension in order to use this kit. The protocol below has been optimized for generating a single-cell suspension from collagenase-dissociated human mammary tissue, but it may be used with a variety of other tissues. Refer to the Product Information Sheet for Collagenase/Hyaluronidase (Catalog #07912) for a protocol for dissociation of human mammary tissue.

GENERATION OF A SINGLE-CELL SUSPENSION

1. Add 5 mL of pre-warmed Trypsin-EDTA (0.25%; Catalog #07901) to the previously dissociated tissue such that the cells are well suspended, and gently pipette up and down with a 1 mL pipette tip for 1 - 3 minutes. The sample should become very viscous due to lysis of dead cells and the release of DNA.
2. Add 10 mL of cold (2 - 8 °C) recommended medium and centrifuge at 450 x g for 5 minutes.
3. Remove as much of the supernatant as possible. The cells may be a viscous mass floating in the recommended medium.
4. Add 2 - 5 mL of pre-warmed Dispase (5 U/mL; Catalog #07913) and 200 µL of DNase I Solution (1 mg/mL; Catalog #07900), and pipette the sample for 1 - 2 minutes. The sample should now be cloudy, but not viscous. If it is still viscous, add more DNase I Solution.
5. Dilute the cell suspension with 10 mL of cold recommended medium and filter through a 37 µm cell strainer (Catalog #27250) into a new 50 mL conical tube (e.g. Catalog #38010) and centrifuge at 450 x g for 5 minutes.
6. Keep cells on ice until ready for use.

Recommended Medium

HBSS with 10 mM HEPES, Without Phenol Red (Catalog #37150) containing 2% fetal bovine serum (FBS).

Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human EpCAM Positive Selection Kit II Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	**Prepare sample at the indicated cell concentration within the volume range.**	1 x 10^8 cells/mL 0.1 - 2 mL* NOTE: If starting with fewer than 1 x 10^7 cells, resuspend cells in 0.1 mL	1 x 10^8 cells/mL 0.25 - 8.5 mL* NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend cells in 0.25 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add Selection Cocktail to sample.**	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 20 minutes	2 - 8°C for 20 minutes
3	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
4	**Add RapidSpheres™ to sample.**	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes
5	**Add cold recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.**	Top up to 2.5 mL	• Top up to 5 mL for samples < 1 mL • Top up to 10 mL for samples ≥ 1 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
6	**Pick up the magnet, and in one continuous motion invert the magnet and tube,** pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.**	Discard supernatant	Discard supernatant
7	**Repeat steps as indicated.**	Steps 5 and 6, three more times (total of 4 x 5-minute separations)	Steps 5 and 6, three more times (total of 4 x 5-minute separations)
8	**Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.**	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

* To minimize cell aggregation, use cold buffers and keep cells on ice as much as possible. If sample begins to aggregate, add DNase I Solution (1mg/mL).

** Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 2 for detailed instructions regarding the RoboSep™ procedure.

Table 2. RoboSep™ Human EpCAM Positive Selection Kit II Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.25 - 8.5 mL NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend cells in 0.25 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	• Human EpCAM Positive Selection II 17846 - High Purity • Human EpCAM Positive Selection II 17846 - High Recovery
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
5	Unload the carousel when the run is complete. Remove the tube containing the isolated cells and resuspend in desired medium. Be sure to collect cells from the sides of the tube.	Isolated cells are ready for use

Notes and Tips

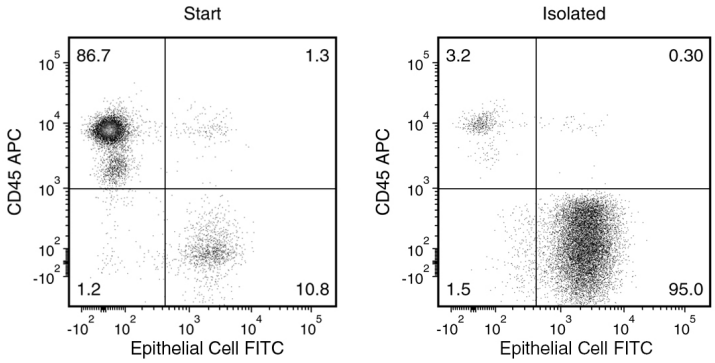
ASSESSING PURITY

For purity assessment by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human Epithelial Cell Antibody, Clone 5E11.3.1 (Catalog #60147), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

NOTE: The 5E11 epitope has an identical distribution to EpCAM.

Data



Starting with peripheral blood mononuclear cells (PBMCs) seeded with MCF-7 cells (breast cancer cell line) at a starting frequency of 10.8 - 50.0%, the EpCAM+ cell content (epithelial cell+CD45-)of the isolated fraction is typically 96.2 ± 3% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 10.8% and 95.0%, respectively.